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Detection of the Cystic Fibrosis Carrier in the General Population: A Pilot Study

L. Picci, M. Cameran, D. Marzenta, O. Marangon, F. Zacchello, M. Scarpa
Department of Pediatrics University of Padova, Italy

Cystic Fibrosis (CF) is the most frequent autosomal recessive disorder in the Caucasian population. The responsible gene (CFTR) maps on Chromosome 7 and since its identification more then 1000 mutation were identified. In the general population, 1: 2500-3000 life newborn is affected, the carrier frequency is estimated as 1:25-27 people. Identification of the healthy carrier in the population remains one of the major issue in the potential planning for a genetic-based identification of couple at risk. Therefore, the major aim of the study is to verify, for the first time in Italy, the feasibility of testing for CFTR mutations on an italian population sample. The study spanned a period over 1995-2004. By the set up of a two-branch semi-automated system, we have analysed 42.000 voluntary healthy subjects with no family history of CF. Among them, 38.000 subjects were coupled and 4000 single. As first branch, all the subjects were analysed by an in-house Reverse Dot Blot detection test developed for the most frequent 48 mutations in the literature. Subsequently, as second branch, negative partners to RDB were analysed by DGGE and eventually sequenced. By these means we were able to assess the frequency of CFTR mutations in our sample population, DF508 having a 41.3% frequency. We have confirmed that 4% of our sample population was carrier for CF mutations, furthermore, we have identified 11 adult subjects compound-heterozygote for CF, suffering for CBAVD. Moreover, we detected 91 couple at risk and performed prenatal diagnosis on each of them.
In conclusion, we can speculate that by coupling RDB and DGEE or DHPLC techniques a genetic testing of the general population might be feasible.

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Reproducibility of nasal potential difference measurements in CF

Y. Yaacov[1], E. Kerem[1], Y. Yahav[2], H. Blau[3], J. Rivlin[4], L. Bentur[5], E. Picard[6], M. Aviram[7], M. Wilschanski[1]
[1]CF Center, Hadassah University Hospital, Jerusalem, [2]CF Center, Sheba Medical Center, Tel Hashomer , [3]CF Center, Schneider Children's Hospital, Petach Tiqva, [4]CF Center, Carmel Medical Center, Haifa, [5]CF Center, Rambam Medical Center, Haifa, [6]CF Center, Shaare Zedek Medical Center, Jerusalem, [7]CF Center, Soroka Medical Center, Beer Sheva, Israel

Aims: Nasal Potential Difference (NPD) measurement has been advocated as a diagnostic tool for CF patients with unusual manifestations and as a method for assessing response to new therapies directed at correcting CFTR function. The purpose of this study is to examine the reproducibility of nasal potential difference measurements.
Methods: 69 patients aged 16 ± 8 years of age (range: 6-52) underwent NPD measurements on at least of two occasions.
Results: 25 patients with classical CF aged 21 ± 8 years of age and 44 patients with atypical CF aged 14 ± 8 years of age underwent repeated NPD measurements. The basal PD and the response to amiloride (Δamil) and response to Cl^- free & isoproterenol (Δchlor) were very similar in both measurements. In the classical CF group the values were -40 ± 12mV vs. -39 ± 11mV (p=0.565) for basal PD, 27 ± 9mV vs. 26 ± 10mV (p=0.549) for Δamil and 2.1 ± 3.8mV vs. 0.4 ± 2.9mV (p=0.069) for Δchlor. In the atypical CF group the values were -32 ± 13mV vs. -28 ± 10mV (p=0.007), 19 ± 10mV vs. 17 ± 8mV (p=0.358) and -3.2 ± 4.6mV vs -3.3 ± 4.4mV (p=0.859), respectively.
Conclusion: When performed in a single center, NPD measurement is a reproducible test for CF patients with classical disease and may be a useful outcome measurement for assessment of efficacy of new treatments. In patients with atypical disease basal PD may vary. This might follow the variability in sweat chloride concentration observed previously.

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Limits of sweat conductivity determinations with Nanoduct® System for rapid sweat testing in patients with Cystic Fibrosis

M.-C. Desax[1], J. Barben[2], R.A. Ammann[1], M. Nelle[1], M.H. Schöni[1]
[1]Department of Pediatrics, University Children`s Hospital of Berne and [2]Pulmonology, Children`s Hospital, St. Gallen, Switzerland

Introduction: Determination of sweat conductivity with the Nanoduct® System is an alternative to conventional chloride measurements.
Methods: With Nanoduct® electrodes conductivity is measured in microprobes (>3µl) on skin and results are available within minutes. We used this test system in a partly selected number of children and some adults with or without CF (n=111; data from St. Gallen, J. Pediatr 2005;146:183-8), in 49 babies (Berne), and in children with symptoms suspected to have CF (n=119; Berne).
Results: In 49 babies (median age: 38.5 weeks gestational age [GA], range 32.4 to 43) determinations were done at a median postpartal age [PPA] of 1.4 weeks (range 0.1 to 21). Conductivity determination was successful in 26 babies (48%). In 230 subjects (3 weeks to 60 yrs) 21 children had no sweat and 14 insufficient sweat to compare Nanoduct® conductivity to conventional chloride. 21 had CF diagnosed earlier, all were identified by the Nanoduct®. Ten times the test was not successful due to skin problems and 13 times due to other clinical conditions (post prematurity 4 x, in 9 cases no reason or technical problems). In 230 patients older than one month Nanoduct® was not successful in 21 subjects (failure rate 9 %). In newborns and prematures the rate of success was lower and significantly associated with GA ($p = 0.003$), PPA ($p < 0.001$), corrected GA ($p < 0.001$), weight ($p = 0.002$), and body temperature ($p = 0.012$).
Conclusion: The Nanoduct® system is a simple rapid test for CF. However, in prematures and newborns as well as in more severely ill children and patients with skin problems the failure rate is higher than previously assumed.

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Sweat conductivity and quantitative pilopcarpine iontophoresis tests for sweat chloride

S. Salomonsson, B.M. Jakobsson, L. Hjelte
Stockholm CF Center, Karolinska University Hospital Huddinge, Stockholm, Sweden

Sweat testing has been the most widely used diagnostic test for cystic fibrosis for almost 50 years. The role of conductivity in definitive CF diagnosis has still to be confirmed.
Objective: To compare the quantitative pilocarpine iontophoresis test for sweat chloride with the Nanoduct conductivity system.
Subjects: Subjects (n=234) with clinical suspicion of CF referred for sweat test were consecutively tested. The subjects were divided into 9 groups by age (0-6 m, 7-12 m, 1-2 y, 3-6 y, 7-12 y, 13-18 y, 19-30 y, 31-50 y, > 51 y).
Methods: Sweat tests were performed using the Nanoduct conductivity system and the standard Gibson and Cooke technique of pilocarpine ionthophoresis simultaneously on the right and left arm, respectively, or on one arm and on the back. Those with a chloride concentration > 60 mmol/L were considered as CF patients (n=11).
Results: The correlation between sweat chloride and conductivity was r=0.67 (p<0.001). The conductivity mean values in non-CF subjects were for each age group: 28, 33, 32, 35, 36, 35, 40, 39, 42 and the mean chloride concentration ones were: 14, 16, 20, 17, 17, 17,21, 23, 27. Of the 11 CF patients five showed a chloride concentration value above the conductivity one. Furthermore, of those with chloride concentrations < 60 mmol/L 14 showed values above the conductivity one, 10 of whom being < 3 years of age. In four of the 14 subjects borderline values would have been missed and in one case possibly a CF diagnosis.
Conclusion: The sweat conductivity method showed good correlation with the quantitative pilocarpine iontophoresis test for sweat chloride. However, before being used as a screening or confirmative method more data is needed concerning cases with higher sweat chloride concentrations than conductivity values.